An ultraspecific anti-SSEA-4 monoclonal antibody recognises stem memory T cells

J Chua[1], E Cid[3], M Vankemmelbeke[1], R McIntosh[1], R Metheringham[1], I Daniels[1], V Brentville[1] and L Durrant[1,2].

[1]Scancell Holdings plc, Oxford UK, [2]Nottingham University, Nottingham, UK, [3]Josep Carreras Leukaemia Research Institute, Barcelona Spain.

Introduction

Stem memory T cells (TSCMs) are the least differentiated population of antigen-experienced memory T cells. They have the ability of self-renewal and multipotency. TSCMs possess higher proliferative, greater survival responses and superior anti-tumour capacity compared to central memory (TCM) and effector memory (TEM) T cells, which make them an attractive candidate for adoptive immunotherapies. However, clinical exploitation of TSCMs is challenging due to their low frequency in blood (2-4% of total T cells) and lack of protocols for isolating and expanding these cells.

The 2811 mAb is an ultraspecific mAb against SSEA-4. The 2811 mAb recognises human and mouse TSCMs and induces T cell proliferation and differentiation *in vitro* and *in vivo*. We demonstrate that 2811 mAb able to identify, isolate and expand TSCM-like cells *in vitro* and *in vivo.*

2811 mAb specificity

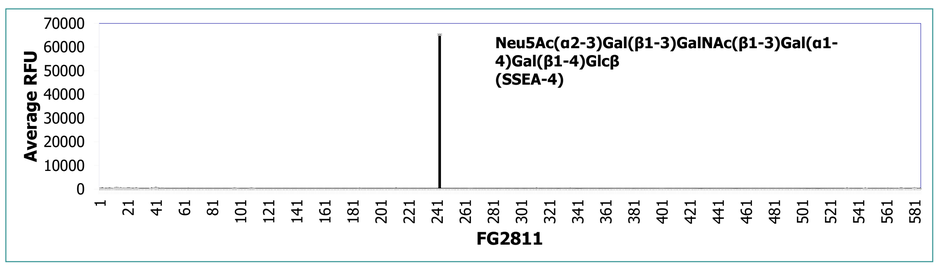


Figure 1. Assessment of 2811 mAb specificity.

Binding of 2811 mAb to 585 glycans on Consortium for Functional Glycomics glycan array. **The 2811 mAb is ultraspecific against SSEA-4.**

2811 mAb binds to PBMCs

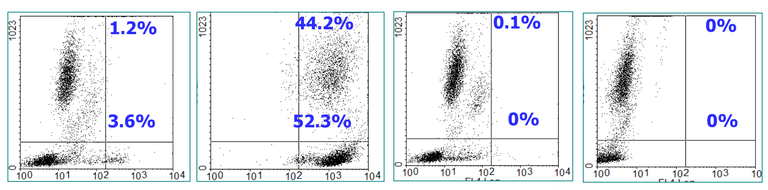


Figure 2. The 2811 mAb bound to PBMCs.

Binding of healthy donor whole blood with 2811 mAb was assessed by indirect immunofluorescence staining and flow cytometric analysis. Anti-HLA-A,B,C mAb (positive), isotype control ab and secondary antibody alone (negative) were used as controls (result is representative of 7 donors). **The 2811 mAb binds 0.8-2.3% of PBMCs (7 healthy donor whole bloods).**

2811 mAb recognises human TSCMs

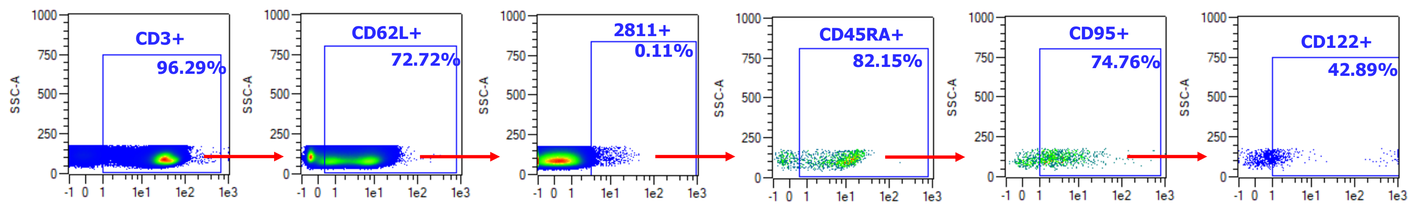
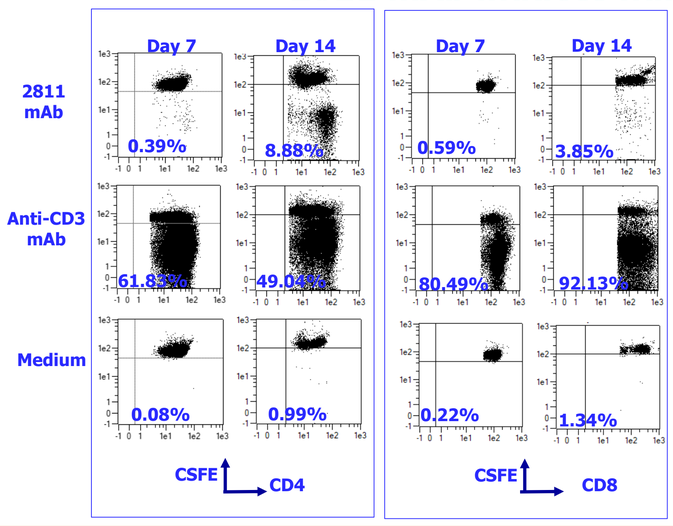


Figure 3. The 2811 mAb recognised TSCMs.

Successive panels depicting the flow cytometric gating strategy used to phenotype CD3/CD62L/2811⁺ T cells. The CD3/CD62L/2811⁺ T cells were checked for the expression of CD45RA, CD95 and CD122 markers. (result is representative of 3 donors).

2811 mAb induces human T cell proliferation *in vitro*



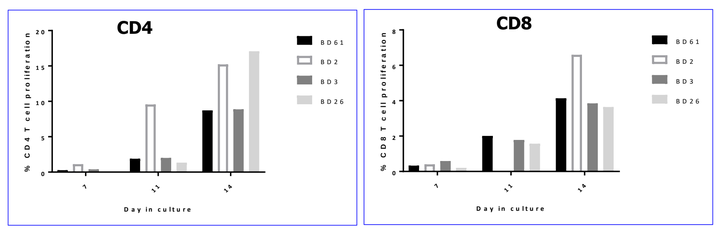
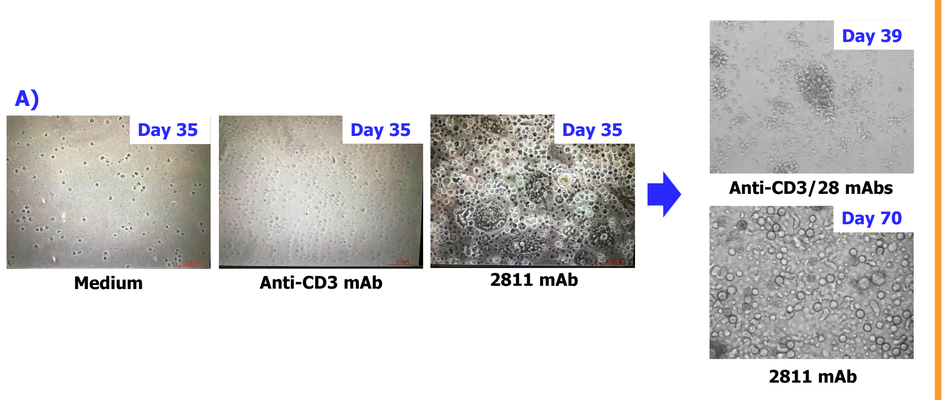


Figure 4. Plate bound 2811 mAb induced T cell proliferation.

CSFE labelled T cells from healthy donors were stimulated with plate bound 2811 mAb, anti-CD3 mAb and medium; cells were collected at day 7, 11 and 14 to check for CD4 and CD8 T cell proliferation. Percentages of specific T cell population proliferation were assessed via CSFE dye dilution analysis (result is representative of 4 donors).

2811 mAb stimulated T cells remains viable *in vitro* for more than 2 months



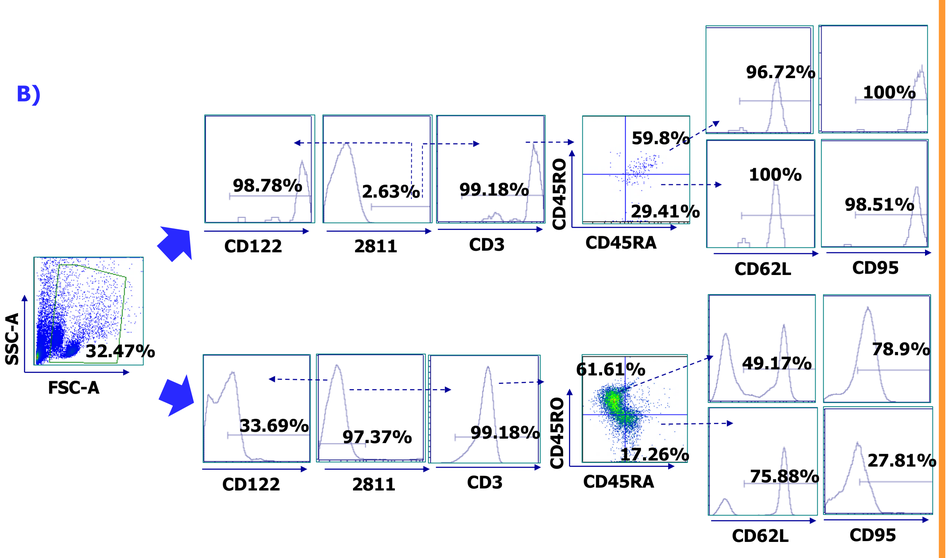


Figure 5. 2811 stimulated T cells remained viable *in vitro* for more than 2 months without any exogeneous cytokines

A, At day 35, only 2811 mAb stimulated T cells remained viable in culture. These viable cells were restimulated with anti-CD3/28 mAbs or 2811 mAbs. T cell blasts were seen in culture restimulated with anti-CD3/28 mAb indicating that these viable cells maintained their proliferative capacity. The 2811 mAb restimulated cells proliferated at slower rate and remained viable more than 2 months in culture.

B, Flow cytometric gating strategy to phenotype viable cells at day 35. Gates were drawn for analysis on 2811⁺ and 2811⁻ cells; they were checked for CD3 and CD122 expressions. The CD3⁺ cells were further checked for the expression of CD45RA, CD45RO, CD62L and CD95 markers.

Self-sustaining cytokines for 2811+ T cells

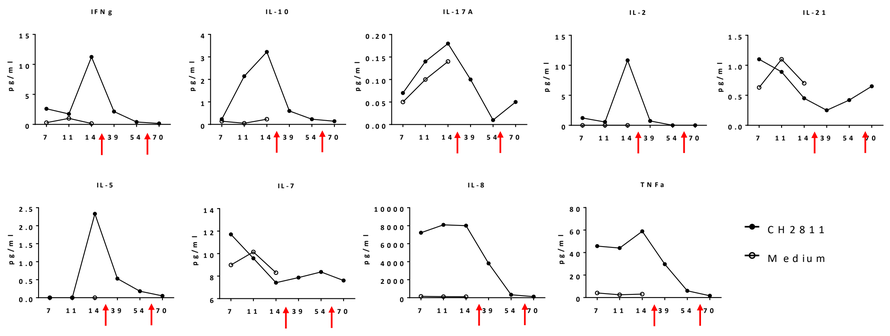


Figure 6. IL-7 and IL-21 could be self-sustaining cytokines for 2811+ TSCMs.

Representative cytokine/ chemokine expression levels (pg/ml) in 2811 mAb stimulated T cells. T cells were stimulated with CH2811 at day 0 followed by restimulation at day 33 and day 64. Supernatants were collected at day 7, 11, 14, 39, 54 and 70 and assessed for the concentration of cytokines/chemokines(pg/ml). Arrows depicted antibody restimulation day.

TCR repertoire diversity analysis

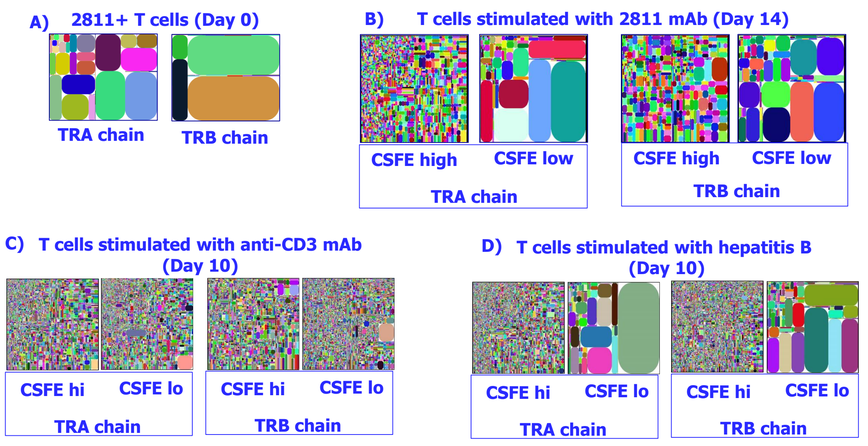


Figure 7. Assessment of TCR repertoire clonotype

T cell repertoire is detected from the extracted RNA of A) 2811+ T cells and CSFE high and low B) 2811 stimulated, C) anti-CD3 stimulated and D) hepatitis B stimulated T cells from healthy donors.

TCR repertoire diversity is illustrated in tree maps where each rounded rectangular represents a unique entry: V-J-uCDR3 and the size of the spot denotes the relative frequency.

Transcriptomic analysis of 2811+ T cells

Stem cell type	Overlapping genes	Adjusted P value
Embryonic	257	0.008
Hematopoietic	103	2.092E-4
Mesenchymal	16	0.061
Embryonal carcinoma	78	3.74E-5

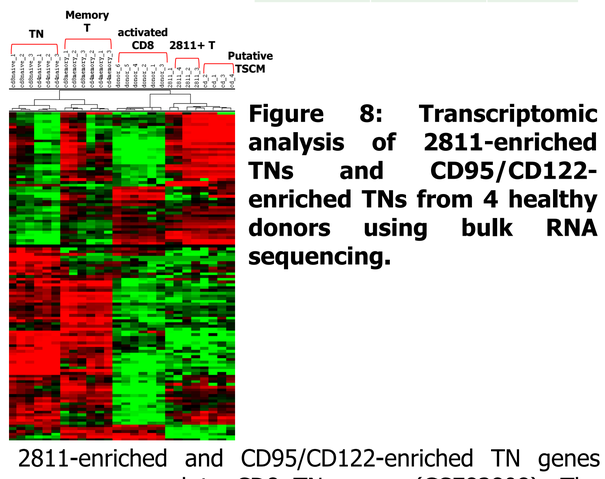


Figure 8: Transcriptomic analysis of 2811-enriched TNs and CD95/CD122-enriched TNs from 4 healthy donors using bulk RNA sequencing.

2811-enriched and CD95/CD122-enriched TN genes were compared to CD8 TN genes (GSE83808). The common differential expressed genes (2227 genes) were analysed for stemness signatures using StemChecker.
Heatmaps and hierarchical clustering of 2811-enriched and CD95/CD122-enriched transcriptomic profiles, based on the 257 overlapping genes from the ESC and 103 from the HSC, respectively.

T cell agonistic effect *in vivo*

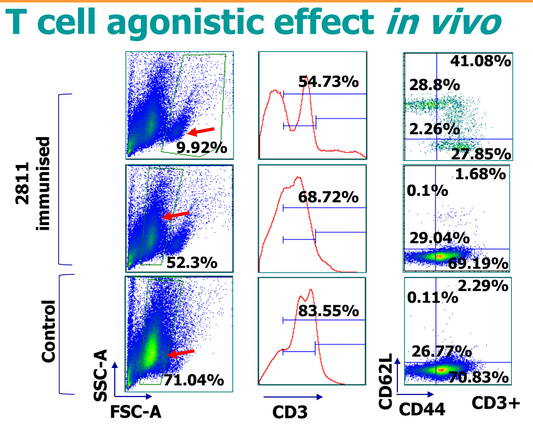


Figure 9. 2811 mAb T cell agonistic effect *in vivo*. C57/B6 mice were immunised with 2811 mAb at day 0. At day 16, splenocytes were harvested and cultured *in vitro* for 7 days without any exogeneous cytokines, followed by phenotyping.

Conclusion: SSEA-4 is a novel marker for human and mouse TSCMs. 2811 mAb can identify and isolate TSCMs and potentially expand TSCMs *in vitro* and *in vivo*. The 2811+ TSCMs are potential candidates for genetic manipulation to express TCRs or CARs and the mAb as agonistic mAb.